Chapter 12

# Models to Evaluate the Prebiotic Potential of Foods

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**Abstract**

The interest in studying the prebiotic effect of foods is increasing due to the way in which the consumption of these foods influences the gut microbiota and how the metabolic activity of the microbiota affects the health and well-being of the host. Several in vitro and in vivo studies have been developed to elucidate the prebiotic effect of foods, and particularly in in vivo studies, the physiological dynamics of this effect has been studied in healthy or diseased individuals. In this chapter, the main in vitro and in vivo models developed for the study of the prebiotic potential of foods will be approached, which can be used by those planning to advance in this field of research.

**Keywords:** functional foods, prebiotics, chronic diseases, animal models, intestinal microbiota

## 1. Introduction

Modern society has changed its standard of living every decade and today, health is becoming an increasingly important personal and social value. Prevention of health problems is prioritized due to the costs associated with curative medicine, especially chronic diseases, which can be prevented by a healthier lifestyle [1]. In addition to the practice of physical activity, adequate nutrition is an essential aspect influencing a person's health status. Consumers are more aware that their food choices can have consequences for their health and maintenance of a healthy lifestyle [2, 3].

Food matrixes are composed of several nutrient or non-nutrient substances that interact in a complex way. In this perspective, foods have the basic function of feeding, some of which present health benefits that go beyond nutrition, such as functional foods. Functional foods may exert physiological benefits and/or reduce the risk of chronic diseases, in addition to basic nutritional functions, and may be similar in appearance to conventional foods and consumed as part of a regular diet [4].

Prebiotics are among functional foods, which are defined as a component of the edible product, in which its health benefit must be measurable and not due to its absorption in the blood stream or due to the sole action of the component, but it should be evidenced that the simple presence of the prebiotic component and the formulation in which it is inserted alter the composition or activity of the microbial flora in the target host by modulating it [4], for stimulating the proliferation of a select group of beneficial colon bacteria and suppressing the proliferation of micro-organisms harmful to health [5].

To be considered prebiotic, food or its components must: (i) resist the processes of host digestion, absorption, and adsorption; (ii) be fermented by the microbiota that colonize the gastrointestinal tract (GI); and (iii) selectively stimulate the growth and/or activity of one or a limited number of bacteria within the gastrointestinal tract, altering the colonic microbiota in favor of a healthier composition [3, 4].

Prebiotics found in natural sources such as vegetables, roots, fruits, milk, and honey are non-digestible carbohydrates such as resistant starch (RS), galacto-oligosaccharides (GOS), fructooligosaccharides (FOS), xylooligosaccharides (XOS), pectic oligosaccharides (POS), and various oligosaccharides that provide carbohydrates fermentable by the beneficial colon micro-organisms [6, 7]. Among these, probiotic micro-organisms such as bacteria belonging to the genus *Lactobacillus* and *Bifidobacterium*, as well as *Streptococcus*, *Saccharomyces cerevisiae*, *Escherichia coli,* and *Bacillus* spp. stand out, which have been studied on a smaller scale. These bacteria are fermentative, obligatory, or facultative anaerobes, and their inherent biological characteristics allow them to prevail over potential pathogenic micro-organisms in the digestive tract [8].

Probiotic micro-organisms are currently defined as live micro-organisms, which when consumed in adequate amounts provide a positive health effect on the host [9]. Butel [10] suggests three modes of action of probiotics, which influence the host's health. One of the first suggested modes of action is called "barrier" effect or resistance to colonization against pathogenic bacteria due to the production of broad-spectrum inhibition bacteriocins, metabolites such as acid lactic and short-chain fatty acids—SCFA (e.g., acetate, butyrate, propionate)—which induce a decrease in pH, being favorable for bacterial growth, or biosurfactants with antimicrobial activity. The improvement of the barrier function in the gut mucosa may be due to the increase of the mucus layer or to the production of defensins and proteins of tight junctions.

In addition to prebiotic and probiotic foods, symbiotic foods, in which probiotic and prebiotic are combined, have been increasingly developed due to the favorable adaptation of the probiotic to the prebiotic substrate before consumption, which may increase the beneficial effects of each of them [11, 12].

In this context, the modulation of the gut microbiota by diet has been studied [13, 14]. The composition and metabolism of the colonic microbiota can be influenced by the type of diet, nutrient balance (mainly carbohydrates, proteins, and fats), and the amount of diet ingested [15]. The impact of diet on microbiota composition is determined by tolerance of gut conditions and by the competition for substrates among microbial species, which demonstrate different capabilities to utilize dietary substrates, promoting the competition for substrates available in the large intestine, playing an important role in defining microbiota composition [16]. The healthy microbiota can be defined as the normal microbiota that maintains and promotes well-being and absence of diseases, especially of the gastrointestinal tract. The colon is the most densely populated part of the gastrointestinal tract and houses about 500 different bacterial species. These bacteria, each with its own spectrum of metabolic activities, make the colon the most metabolically active organ in the human body [17].

The gut microbiota influences the metabolic processes, preventing and modulating chronic diseases such as obesity, diabetes, insulin resistance, and cardiovascular diseases [18] because it interferes in several systems such as cardiovascular [19], nervous [20, 21], immune [22], endocrine [23], and the gastrointestinal system itself.

From this perspective, the prebiotic effect of foods can be studied from in vitro systems or from in vivo models using healthy and diseased animals or humans. Each model has advantages and disadvantages, which will be discussed in the next sections of this chapter.

## 2. Types of prebiotics

Dietary fibers (DF) are bioactive components, which may have prebiotic activity, present in plants, defined as the edible part of plants or analogous carbohydrates resistant to digestion and absorption in the small intestine of humans, with complete or partial fermentation in the large intestine [24, 25]. Regarding water solubility, DFs are classified as soluble (SDF) and insoluble (IDF). IDFs include cellulose, lignin, and some hemicelluloses and pectins [26, 27]. SDFs, however, comprise the majority of pectins, gums, mucilages, and hemicelluloses [28, 29].

The concept of DF has been expanded to include functionally similar substances such as RS, inulin, FOS, and GOS. GOS or FOS may have beneficial effects such as anti-adhesion or direct immunomodulation that do not require fermentation and are therefore called additional biological activities not related to their effects on the gut microbiota [30]. There are several prebiotics with various origin and chemical properties. Inulin, FOS, GOS, lactulose, and polydextose are recognized as established prebiotics, whereas isomaltooligosaccharides (IMO), XOS, and lactitol are categorized as emerging prebiotics. In addition, resistant starch-rich whole grains are considered prebiotic in nature, and it is assumed that their consumption leads to many health benefits [31]. The fermentability of dietary fibers such as oat b-glucan, flaxseed gum, and fenugreek gum suggests their potential prebiotic

application in promoting human health [31]. The main technological applications of prebiotics and the potential beneficial health effects on consumers of these foods are described in **Figure 1**.

Plant-derived polysaccharides arrive unchanged in the colon, being degraded by micro-organisms living in the human GI tract to SCFA (**Figure 2**). The degradation of complex oligosaccharides (pectin, cellulose, hemicellulose, and resistant starches) involves a strong metabolic alignment among diverse micro-organisms that makes up the intestinal microbiota, but these mechanisms are still not fully understood [24, 32].

In addition to DF, phenolic compounds (PC) or polyphenols may also benefit the gut microbiota, as up to 90% of plant PCs reach the colon and are used as substrates for the microbial production of small phenolic acids [33]. In turn, these biotransformed compounds modulate the microbial population in the gastrointestinal tract and are used as substrates for the production of SCFA [33, 34]. Results have reported that there is a possible interference of PC in

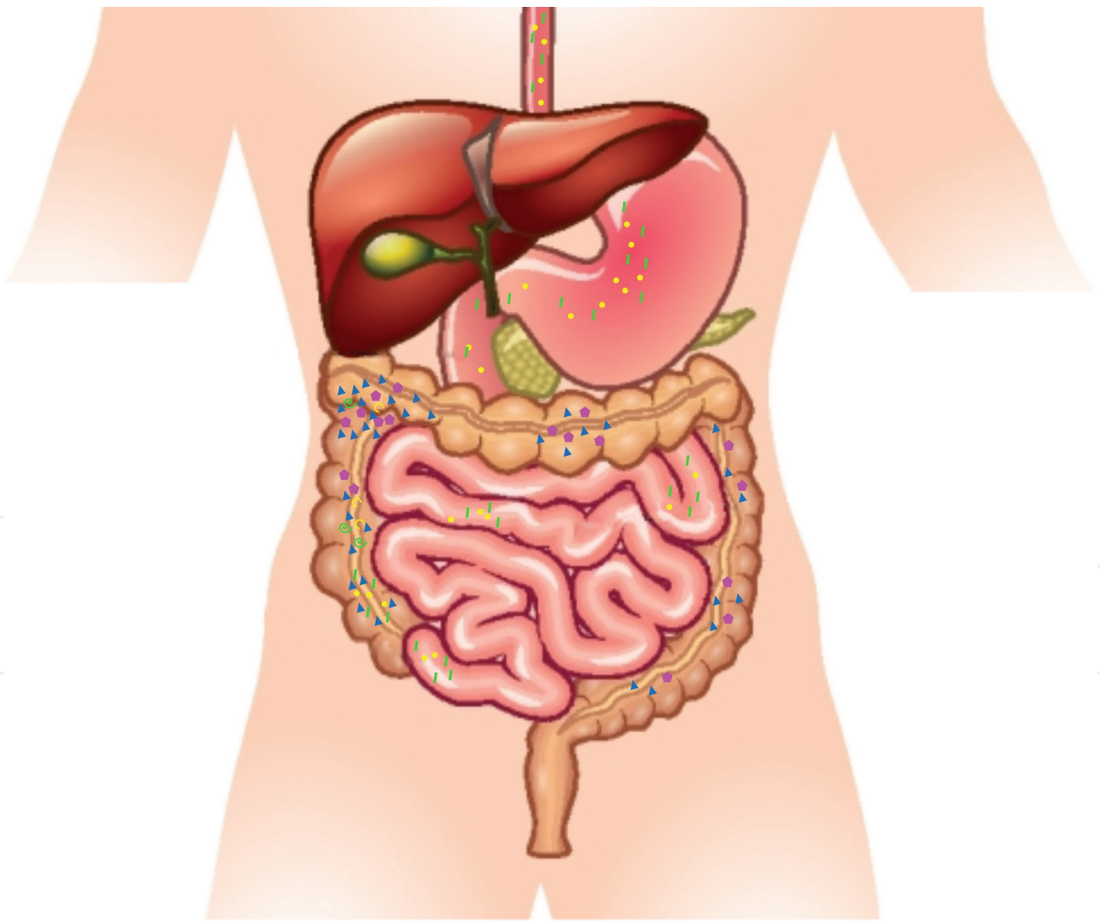

**Figure 1.** Degradation of dietary fibers and phenolic compounds by the gut microbiota. Dietary fibers (/) and phenolic compounds (●) reach the colon (mainly in the proximal part) and suffer a primary degradation by bacteria (▲) to oligosaccharides and monosaccharides (ᔓ) and small phenolic acids (○), respectively. Then, these compounds are used by the gut microbiota for the production of SCFA (⬟), which increase the number of beneficial intestinal bacteria.

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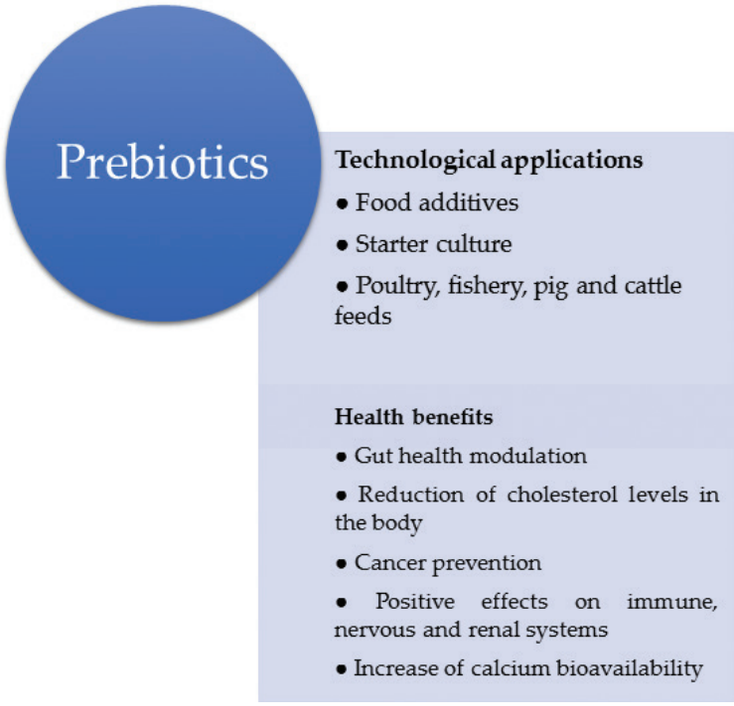


**Figure 2.** Some technological applications of prebiotics and health benefits from consumption.

the increase of viable *Bifidobacterium* and *Lactobacillus* cells in the intestine (in vivo model) and feces of animals or humans (in vitro model) [35, 36].

PC are secondary metabolites derived from pentoses-phosphate, shikimic acid, and phenylpropanoid pathways in plants. They are divided into four main classes according to their chemical structure: flavonoids (including flavonols, flavanols, flavanones, flavones, anthocyanidins, chalcones, dihydrochalcones, dihydroflavonols, and isoflavones), lignans, stilbenes, and tannins. They have numerous reported physiological properties, such as vasodilators, anti-thrombotic, anti-inflammatory, anti-apoptotic, hypolipemic, or anti-atherogenic properties [37].

Prebiotics should be ingested daily as a way of ensuring continuous effect on the intestinal microbiota. However, recommendations for daily doses will depend on the type of food containing the prebiotic compound (naturally or added) or the isolated prebiotic compound consumed as a nutraceutical or prebiotic administrated by gavage (orogastric) or added to diet. The consumption of 5–8 g per day of inulin, FOS, or RS has been shown to significantly increase fecal bifidobacteria [38, 39]. In another study, rats received daily oral administration (gavage) of FOS (3 g/kg) or GOS (4 g/kg) for 5 weeks [40].

Other studies have added prebiotics to diets for rodents such as Sprague-Dawley rats that consumed a high-fat diet and diet added of 10% oligofructose [41] or rats that consumed AIN-G diet added with 10% inulin or oligofructose [42]. Healthy or diabetic Wistar rats consumed basal diet supplemented with XOS (10%) or FOS (10%) or a combination of XOS (5%) and FOS (5%) [43].

## 3. Use of in vitro models in the study of the prebiotic potential of foods

In vitro modeling is useful for investigating the prebiotic potential of foods as it is less expensive, does not require sophisticated handling techniques, and allows simulating fermentation processes that occur along the large intestine and have few ethical limitations. However, they present limitations such as absence of interaction between neuroendocrine and immunological systems with the microbiota; absorptive processes, secretions, and defense systems are not incorporated into the models, as well as difficulty in controlling changes in the structures of microbial communities after inoculation. In these studies, it is possible to use pure microbial populations, known mixtures or fecal material [44].

The groups of colon bacteria present selective characteristics regarding the substrates available, and it is recommended that the studies use the mixed microbial culture, which simulates the microbial ecology of the human intestinal tract. Fermentation in anaerobic batches inoculated with fecal suspensions provides an excellent mode for small-scale screening of new substrates. Until recently, the growth of specific bacteria in such fermentations was measured by counting colonies on selective agar. This approach, however, has several disadvantages (time-consuming, labor intensive, and non-recovery of uncultivable organisms). As a result, molecular techniques such as fluorescence in situ hybridization (FISH) were developed to study microbial communities [13, 45]. FISH involves the use of genus-specific and in some cases species-specific fluorescently labeled oligonucleotide probes. Hybridization of the probe that has its own specificity to recognize a particular group of bacteria to the complementary target sequence within bacterial cells results in fluorescently labeled cells that can be visualized and enumerated using fluorescence microscopy [45].

Generally, food or a substrate prebiotic extracted from the test food itself is lyophilized and supplemented in different concentrations to Man, Rogosa and Sharpe (MRS) medium; the negative control is represented by the MRS medium without the addition of the test food or substrate, and the positive control is represented by inulin [46, 47] or fructooligosaccharide [17, 48], which are recognized prebiotics. Frequently, experiments include the MRS medium with addition of glucose as the carbon source, which also serves as a control. After media are defined, probiotic micro-organism strains such as *Lactobacillus* or *Bifidobacterium* are incubated and the samples are incubated under ideal conditions for the selected micro-organisms. Thereafter, viable cell counts and metabolism monitoring of these micro-organisms (quantification of short-chain fatty acids and pH, among other parameters) are performed to confirm the prebiotic property of the food [47, 49]. SCFAs are saturated aliphatic organic acids that have from one to six carbon atoms, such as acetate (C2), propionate (C3), and butyrate (C4), and are the final products of bacterial fermentation processes.

Recently, many byproducts of the food industry have been studied as cheap and alternative sources of prebiotics [6, 49, 50]. The prebiotic effect of cashew apple (*Anacardium occidentale* L.) agro-industrial byproduct powder on different potentially probiotic Lactobacillus strains (*L. acidophilus* LA-05 and *L. casei* L-26 and *L. paracasei* L-10) was cultivated in broth containing cashew apple powder (20 or 30 $g.L^{-1}$), glucose (20 $g.L^{-1}$), or FOS (20 $g.L^{-1}$). The cell viability of Lactobacillus strains (counts of viable cells) and changes in pH values, production of organic acids, and consumption of sugars in growth media were monitored for 48 h. The cultivation

of *Lactobacillus* strains in broth containing glucose, FOS, or cashew apple powder resulted in high counts of viable cells, decreased pH, production of organic acids, and consumption of sugars over time, revealing intense bacterial metabolic activity and prebiotic activity [50]. Thuaytong and Anprung [51] used 1% (v/v) of prepared *L. acidophilus* LA-5, and *Bifidobacterium lactis* BB-12 was transferred into MRS broth, which was composed of 1% (w/v) glucose or 1% (w/v) inulin or 1% (w/v) prebiotic (guava samples), and demonstrated that both red guava and white pulp induced similar growth of prebiotic bacteria in glucose-containing medium.

The study by Gómez et al. [49] confirmed the prebiotic effects caused by a refined product containing POS that promoted the growth of beneficial bacteria and the increase of SCFA concentrations. In a study carried out by Sousa et al. [52], yacon flour revealed a potential prebiotic activity in the growth of probiotic strains *Enterococcus faecium* 32, *Bifidobacterium animalis* Bo, *L. acidophilus* Ki, and *L. casei* L26, probably due to its content in FOS. Teixeira et al. [47] evaluated the influence of Amazonian tubers *Dioscorea trifida*, *Calathea allouia,* and *Dioscorea altissima* on the growth of *Lactobacillus acidophillus* bacteria and observed that the best in vitro result was for *D. trifida* fiber, which stimulated the bacterial growth without significant difference from commercial inulin.

Another in vitro model that is being used to evaluate the prebiotic activity of foods is the fermentation of animal or human feces added to the test food or extract [13, 53] and it is also used for the purpose of evaluating the metabolism of fecal micro-organisms.

The beneficial health effects of prebiotics are related to their influence on the gut microbiota composition, stimulation of growth, metabolism, and activities of lactic acid bacteria, bifidobacteria, and other emergent strains such as *Roseburia intestinales* and *Faecalibacterium prausnitzii*) [7].

Quinoa (*Chenopodium quinoa* W.) and amaranth (*Amaranthus caudatus* L.) submitted to in vitro digestion and together with a control (without external carbon source) were used as carbon sources in batch cultures with fecal human inocula. After 48 h of incubation, both substrates stimulated in a similar proportion the growth of certain numerically predominant bacterial groups in the human gut microbiota, including *Bifidobacterium* spp., *Lactobacillus-Enterococcus*, *Atopobium*, *Bacteroides-Prevotella*, *Clostridium coccoides-Eubacterium rectale*, *F. prausnitzii,* and *Roseburia intestinalis* assessed by FISH, in addition to total SCFAs (acetate, propionate, and butyrate) with a decrease in pH, suggesting that these pseudocereals can have prebiotic potential [13].

Broad beans (*Vicia faba*) and lupin seeds (*Lupinus albus*) were submitted to in vitro digestion used as carbon sources in anaerobic batch cultures to evaluate their impact on the gut microbiota composition (by FISH) and on their metabolic products (lactate and SCFAs). The fermentation of the lupine seeds resulted in a higher total amount of SCFA than the bean fermentation, and in both, there was a decrease in the pH of the fermentation medium. In addition, legume fermentation increased microbial fecal batch cultures, such as *Bifidobacterium spp., Lactobacillus-Enterococcus, Atopobium, Bacteroides-Pretovella, C. coccoides-E. rectale, F. prausnitzii,* and *R. intestinalis* [54].

The prebiotic potential of POS obtained by orange peel wastes was assessed by in vitro fermentation using human fecal inocula. For comparative purposes, similar experiments were

performed using orange pectin and commercial FOS as substrates for fermentation. POS particularly increased the amount of bifidobacteria and lactobacilli (assessed by FISH) so that the ratio between the counts of both genera and the total cell number increased from 17 in the inocula to 27% after fermentation. SCFA generation from POS fermentation was similar to that observed with FOS [49].

Sugar beet pulp (*Beta vulgaris* L.) and lemon peel wastes (*Citrus limon* L.) were used to obtain two mixtures of POS and in comparison, FOS and commercial pectins were assessed by in vitro fermentation and FISH using human fecal inocula. The joint populations of bifidobacteria and lactobacilli increased from 19 up to 29, 34, and 32% in cultures with pectic oligosaccharides from lemon peel wastes, beet pulp, and FOS, respectively. *Faecalibacterium* and *Roseburia* also increased their counts with all substrates (especially with pectic oligosaccharides from lemon peel wastes). The highest concentrations of organic acids were observed in media containing oligosaccharides, and these results confirm that pectic oligosaccharides present better prebiotic properties than pectins and are similar or better than FOS [6].

The prebiotic effect of oligosaccharides recovered and purified from caprine whey was evaluated by in vitro fermentation under anaerobic conditions using batch cultures at 37°C with human feces (by FISH). In this research, growth of *Bifidobacterium* spp. was significantly higher with purified oligosaccharides compared to the negative control. Lactic and propionic acids were the main SCFAs produced. These findings indicate that oligosaccharides naturally extracted from caprine whey or cheese whey (byproduct) could be used as new and valuable sources of prebiotics naturally produced in the lactating mammary gland of domestic species

| Food | Main results | References |
|---|---|---|
| Oligosaccharides from Pitaya (*Hylocereus undatus* (Haw.)) | ↑ Resistance to gastric acidity<br>↑ Growth of *Bifidobacterium* and *Lactobacillus* | [56] |
| Byproducts of date pits (*Phoenix dactylifera* L. var.Medjoul) and apple bagasse (*Malus domestica* var. rayada) | Fermentation by colonic bacteria produced AGCC (formate, succinate, acetate, propionate, and butyrate) | [57] |
| Pomegranate peel (*Punica granatum*) | Fermentation of pomegranate peel flour by colonic bacteria generated acetic, propionic, and butyric acids | [36] |
| water-soluble xylan from wheat bran (XOS extraction) | ↑ Growth of *L. brevis, B. adolescentis,* and the *Weissella* spp. on XOS<br>↑ Lactic acid and acetic acid production after 48-h incubation. | [58] |
| raw and roasted almonds (*Prunus amygdalus*) | Predigested raw and roasted almonds promoted the growth of *Lactobacillus acidophilus* (La-14) and *Bifidobacterium breve* (JCM 1192), and no significant differences were found between these two nuts | [59] |
| Apple pectin (*Malus domestica*) | ↑ *Bifidobacterium*, *Lactobacillus,* and *Streptococcus* (including *Enterococcus*) in feces; ↓ *C. perfringens*, enterobacteria and Pseudomonas; ↑ Fecal concentrations of SFCA | [60] |

**Table 1.** In vitro studies on the prebiotic potential of foods.

and not obtained by enzymatic reaction (trans-galactosylation) from lactose, although numerous papers and patents mostly refer to specific GOS [55].

Other studies evaluating the prebiotic potential of food using in vitro models are described in **Table 1**.

## 4. Use of in vivo models to study the prebiotic potential of foods

It has been well established that the colon microbiota has a deep influence on health. The study of the prebiotic potential in humans would be considered as a gold standard in case of absence of ethical and practical limitations, which may make the research unreliable or limited, in addition to the high dropout rates of study participants. Thus, animal models become an alternative to study the prebiotic potential of foods, since they allow direct access to intestinal contents as well as to organs and tissues [61].

Usually, the animal models used for the study of gut microbiota are swine [62], zebrafish [63], and more widely in rodents such as rats [47], hamsters [64], and mice [53], especially when the potential prebiotic of foods is evaluated.

Teixeira et al. [47] confirmed the prebiotic potential of Amazonian tubers by adding them to the diet of Wistar rats for 28 days, evaluating the pH and microbiota present in feces collected from the animals' caecum. Samal et al. [65] evaluated the prebiotic potential of Jerusalem artichoke (*Helianthus tuberosus* L.) added at different concentrations to the diet of rats for 12 weeks and observed that the consumption promoted beneficial effects on immunity, intestinal morphometry, and hindgut fermentation of rats. Supplementation with 2.5% of insoluble fibers from pineapple peel decreased the daily production of fecal ammonia, shortened gastrointestinal transit time, and increased the total amounts of SCFA in the caecal content as well as the growth of gut microflora such as *Lactobacillus* spp. and *Bifidobacterium* spp. in hamsters [64].

Not only should the gut microbiota be evaluated in in vivo studies but also other variables such as pH, feces humidity, and SCFAs production, which is directly related to the selective bacterial fermentation of prebiotics [66, 67]. In the large intestine, 95% of SCFA produced are rapidly absorbed by colonocytes, whereas the remaining 5% are expelled in the feces [68]. These microbial metabolites can be used as sources of energy by the host and can also act as regulators of energy consumption and metabolism [69]. pH acidification can also be an indicator of fermentation of prebiotic components of foods in the colon by endogenous bacteria and production of organic acids directly responsible for this process [70, 71]. In addition, the preservation of the intestinal epithelium in healthy rats or its recovery in diseased rats may provide evidence of the prebiotic potential, as observed by Hu et al. [72] and Moura et al. [73].

Bränning et al. [74] evaluated the potential prebiotic of blueberry husks added in diet as a substitute for digestible starch. The consumption of diet containing blueberry husk by rats for 5 days resulted in higher amounts of propionic acid and butyric acid in the distal colon and feces, respectively, when compared to rats that were fed a control diet without fibers. Both

acids are essential substrates for colonic epithelial cells, improving gut health, and a surplus of substrates which also have metabolic effects. However, blueberry husk has antimicrobial effects, as observed by the decreased counts of lactobacilli, bifidobacteria, and enterobacteriaceae, and the larger pool of succinic acid may be a consequence of these antimicrobial effects. In this model, blueberry husks do not demonstrate prebiotic properties.

Rodríguez-Cabezas et al. [39] evaluated the synergistic effect of two dietary fibers with different fermentation patterns, FOS (Beneo Ò-95) and RS (FibersolÒ-2), administrated to healthy rats or in trinitrobenzenesulphonic acid (TNBS) colitic rats. Treatment groups (n = 20) received FOS (2 g/rat/day), RS (2 g/rat/day), or the mixture of both (37.5 FOS and 62.5% RS) (2 g/rat/day) incorporated in drinking water during 2 weeks. In healthy rats, the administration of the combination of FOS and RS induced changes in the intestinal microbiota and increased lactobacilli and bifidobacteria in caecum and colonic contents. In addition, treatment increased the moisture content and decreased the pH of caecum and colon. Furthermore, its administration upregulated the expression of trefoil factor-3 and mucin 2 (MUC-2) in comparison with untreated rats, thus improving the intestinal barrier function and increasing the propionate, butyrate, and total SCFA colonic contents. The beneficial effects observed with this combination were confirmed in the healthy or colitis rats.

| Study model | Foods | Main results | References |
|---|---|---|---|
| Female rats Wistar | Cocoa fibers (*Theobroma cacao* L.) | ↑Bifidobacterium and Lactobacillus; ↑ SCFA production and ↓ cecal and fecal pH | [76] |
| Male golden Syrian hamsters | Pineapple peel (*Ananas comosus* L. Merr.) | Modulation of the activities of fecal bacterial enzymes; ↓ ammonia contents in caecum and feces; ↑ concentration in the caecum of SCFA | [64] |
| Male rats Wistar | Passion fruit peel (*Passiflora edulis*) | Positive effect on SCFA production, but no change in gut microbiota was observed | [77] |
| Male rats Wistar | FOS and PC of strawberry (*Fragaria ananassa*) | ↓ Cecal pH and ↓ production of putrefactive SCFA (sum of isobutyric, isovaleric, and valeric acids) | [78] |
| Male guinea pigs | FOS of Yacon (*Smallanthus sonchifolius* Poepp. & Endl) | ↑ Cecal SFCA concentration | [79] |
| Male BALB/c mice | GOS of Chinese roots (Deshipu stachyose granules) | Growth of beneficial intestinal bacteria (Lactobacilli and Bifidobacteria) and inhibition of pathogenic bacteria (*Clostridium perfringens*) | [80] |
| | | Effects on intestinal peristalsis promotion and bowel function improvement (constipation treatment) | |

**Table 2.** In vivo models for prebiotic food assessment.

Young adult male rats were fed ad libitum with purified control diet (CONT) containing 5% w/w cellulose (insoluble fiber) or diet containing 10% w/w cellulose (CELL), FOS, oat beta-glucan (GLUC), or apple pectin (PECT) for 4 weeks. Comparing CONT and CELL, caecal concentrations of fermentation products increased from 1.4 to 2.2 times in GLUC, FOS, and PECT, and colonic concentrations increased from 1.9 to 2.5 times in GLUC and FOS; however, no consistent changes in SCFA receptor gene expression were detected. The main fermentation products detected were acetate, propionate, butyrate, and succinate, and the differences in amounts of fermentation products among soluble fibers may reflect different fermentation patterns and/or different fermentation rates and turnover. This research concluded that the presence of soluble fermentable fiber appears to be more important than its source [75].

Other studies evaluating the prebiotic potential of foods using in vivo models are described in **Table 2**.

## 5. Prebiotics and other beneficial effects on health

The modulations of the intestinal microbiota and SCFA production are associated with many beneficial effects about the ingestion of prebiotics and isolated or added to foods, such as regulation of various physiological processes (e.g., inflammation) and metabolic processes (e.g., lipid and glucose metabolism), thus contributing to the treatment or prevention of chronic non-degenerative diseases [38].

Rats treated with prebiotics had a reduction of plasma pro-inflammatory cytokines, reduction of hepatic inflammatory expression, and oxidative stress markers [81]. Everard et al. [82] showed that diet enriched with prebiotics led to an improvement in glucose tolerance, increase in amount of L-cells, and associated parameters (expression of intestinal pro-glucagon mRNA and plasma glucagon-like peptide-1 levels or GLP-1) in addition to reduction in body fat accumulation, oxidative stress, and level of inflammation in obese rats.

Salazar et al. [69] supplemented 15 obese women with a mixture of inulin and oligofructose for 3 months and observed that prebiotics had a bifidogenic effect, but the elimination of SCFA in feces did not show a significant correlation with the serum concentration of lipids.

A prospective longitudinal cohort study with 1592 workers with metabolic syndrome found that there was an inverse association between consumption of insoluble fibers and increase in systolic and diastolic blood pressure, total cholesterol (TC), triglycerides (TG), apolipoprotein B100, and TG/high-density lipoprotein (HDL) ratio; however, the ingestion of soluble fibers was inversely associated only with triglycerides and apolipoprotein B100. Thus, the prevalence of metabolic syndrome was lower in participants who ingested larger amounts of insoluble fibers [83]. In contrast, a meta-analysis by Wu et al. [84] that included 18 cohort studies with 672,408 participants confirmed that dietary intake of soluble or insoluble fibers (especially from cereals and fruits) has a similar inverse effect associated with the risk of coronary heart disease.

Barbalho et al. [85] reported that the supplementation of passion fruit peels to healthy Wistar rats contributed to the elevation of HDL levels and the decrease in glycemia, TG, and TC levels

of these animals compared to the control group. Such results would be associated with the soluble dietary fiber present in passion fruit peels, such as mucilage and pectins, which form a viscous gel that retains water and reduce the sensation of hunger, body weight, plasma levels of TC, TG, and low-density lipoprotein (LDL) and increase the excretion of cholesterol and bile salts in feces and HDL levels.

Obese rats fed with hyperlipid diet and diet added of lyophilized jabuticaba peel (rich in anthocyanins) exhibited increased HDL and improved insulin resistance, suggesting that the diet added of this byproduct may have a protective effect against cardiovascular diseases by increasing HDL levels [86].

Amaya-Cruz et al. [87] evaluated the effect of dietary fibers and polyphenols from guava (*Pisidium guajava*), peach (*Prunus persica*), and mango (*Mangifera indica*) byproducts on obesity-related hyperglycemia and hepatic steatosis in Wistar rats. Mango and peach byproducts presented better soluble/insoluble fiber ratio and high amount of polyphenols, which may have attenuated the development of hepatic steatosis and hyperglycemia in rats. In guava byproducts, they found great amount of soluble dietary fibers and condensed tannins, which may be related to the greater anti-obesogenic effect on animals, when compared to control rats and to those treated with other byproducts.

Changes in the intestinal microbiota may also influence the homeostasis of the immune [35], renal [88], and nervous systems [89], as well as the development and progression of pathophysiological processes such as hypertension [90] and colorectal cancer [91]. A mixture of non-digestible GOS ingested by mice for 3 weeks prior to induction of inflammatory neuropathology and anxiety improved anxiety and inflammation through decreased expression of IL-1b cytokine and 5-HT2AR serotonin receptor in the frontal cortex compared to the control group [92]. Healthy men and women daily supplied with FOS or GOS for 3 weeks showed decreased response to cortisol awakening, protecting against the risk of depression [93]. Rats with chronic kidney disease (CKD) fed for 3 weeks with RS diets had a delay in CKD progression and increased creatinine clearance when compared to CKD mice that received amylopectin [94].

## 6. Innovations in food processing with added prebiotics

The inclusion of prebiotics in industrialized foods has become a viable and healthy alternative, since there is a great demand of consumers for functional foods that can help in maintaining health. Moreover, the food industry can obtain numerous advantages from the addition of prebiotics in food products, such as improvement of sensory characteristics, better balance of the nutritional composition, and longer shelf-life [67]. In general, prebiotics are added to bakery products, breakfast cereals, beverages (e.g., fruit juices, coffee, cocoa, and tea), dairy products, table spreads, butter-based products, and desserts (ice cream, puddings, jellies, and chocolates) [67, 95]. Prebiotics also have gelling properties (e.g., inulin), which maintain the emulsion stability, provide spreadable texture, and water retention (e.g., inulin and FOS), thus allowing the development of processed foods with low fat content, with pleasant taste and texture [67, 96].

However, some important characteristics of the manufacturing process, such as low pH, high temperatures, and conditions favoring the Maillard reaction must be taken into account when

choosing the prebiotic to be added to foods in order to avoid the formation of anti-nutritional compounds detrimental to the sensory quality of the final product and consumer health as well as the partial or total reduction of their action. Among prebiotics commonly used in the food industry, GOS are more stable at high temperatures and low pH mainly due to the beta bonds of their structure, which provide greater hydrolysis stability compared to FOS and inulin [96]. A type of RS known as RS3 can be added to fried battered products to increase the content of dietary fibers and avoid reducing moisture and the absorption of fats, since RS3 is very resistant to frying temperatures [97].

## 7. Concluding remarks

The importance of the consumption of prebiotics is unquestionable and they should be part of healthy diet. Prebiotics exert various technological functions in food and many health benefits not only related to the modulation of the intestinal microbiota but also to other beneficial physiological actions in various organs and systems of healthy or diseased men/animals. In this sense, the development of foods added due to prebiotics by the industry can be advantageous due to the demand and profitability of this market, as well as for consumers who will have healthy foods available that can be readily consumed for the prevention or treatment of diseases, thus reducing public health costs. However, there is no consensus on the recommended quantity of specific prebiotics for consumption in the diet, and this limitation is a major challenge regarding the different in vitro and in vivo models used to test the prebiotic potential of foods.

Both in vivo and in vitro models have helped advances of researches aimed at evaluating the prebiotic potential of foods through the composition and metabolism of the intestinal microbiota and their interactions. However, it is noteworthy that there are no ideal models, and the most adequate are those based on the study objectives and using association of complementary techniques.

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